

# **SQUIRREL MONKEY REQUIREMENTS FOR CHRONIC ACCELERATION**

**FINAL REPORT**

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## **SUMMARY**

This study examined: 1) the ability of a small non-human primate to tolerate chronic centrifugation on a centrifuge with a radius of 0.9 m, and 2) the influence of centrifuge radius on the response of primates to hyperdynamic fields. Eight adult male squirrel monkeys were exposed to 1.5 g via centrifugation at two different radii (0.9 m and 3.0 m). Body temperature, activity, feeding and drinking were monitored. These primates did tolerate and adapt to 1.5G via centrifugation on either radius centrifuge. The results show, however, that centrifuge radius does have an effect on the responses of the primate to the hyperdynamic environment. Adaptation to the hyperdynamic environment occurred more quickly on the larger centrifuge. This study demonstrates that a small, non-human primate model, such as the squirrel monkey, could be used on a 0.9 m radius centrifuge such as is being considered by the NASA Space Station Program.

## INTRODUCTION

Data from spaceflight and ground based experiments have clearly demonstrated the importance of Earth gravity to normal physiological function in man and animals. Understanding the mechanisms of these effects and developing rational and effective counter-measures to astronaut deconditioning in space require the investigation of these gravity-dependent systems and functions under conditions of long-term weightlessness/microgravity. In order to fully understand the nature of the interaction of biological systems and gravity, it is necessary to make repeated observations at several field strengths between 0 and 1 G. A centrifuge in an orbiting spacecraft provides the only method for such studies of these gravity-dependent phenomena between microgravity and Earth gravity. This centrifuge must be capable of accommodating humans, experimental animals and plants -- both intermittently and/or continuously. However, such rotational facilities are not without technical and physical limitations, which may also prove to be severe biological limitations.

For example, G-gradients are inherent in all gravitational fields. In man on Earth there is a calculable head-to-foot G-gradient. The problem of G-gradients in animals on centrifuges is a quantitative, rather than qualitative, one. Centrifuge geometry produces several irregularities in acceleration fields, all of which are inversely related to the size of the radius of rotation. These potentially adverse aspects of centrifugation have not been critically examined. Where rotational radii must be limited, such as in spacecraft, these factors may interfere with research objectives and they must be considered during design of such centrifuges and as a factor in their experimental usage. Conversely, if the size of the centrifuge is severely constricted, the effect of that parameter must be fully understood and experiments designed with those limitations taken into consideration.

In addition to providing an inertial field, centrifuges have other characteristics that may, if not controlled, confuse the gravitational effects [6]. The basic physical relationships of a centrifuge are:

$$a = r\omega^2; \text{ or}$$

$$G = a/g = r\omega^2/g$$

where: **a** is the acceleration (inertial) field;  
**r** is the radius of rotation;  
 $\omega$  is the rotation rate (radians/time);  
**G** is the field characteristic (the weight-to-mass ratio); and,  
**g** is the Earth's gravitational constant.

The reciprocal relationship between radius and rotation rate allows the same field to be developed at infinite combinations of radius and rotation rate. Whether rotation rate has an effect, separate from field strength, has never been adequately determined. In practice, the possibility of such a biological influence has been recognized, and minimized, by utilizing a large radius (usually 2-3 m). However, in the Space Station, centrifuge radius will be limited and any possible separate rotatory influence must be identified. It is crucial that any interaction between rotation rate and G-field be thoroughly explored; such information may indicate the maximum fields (for a given radius) that do not develop interference from rotatory effects.

In this research program our intent was to determine if there is an influence of centrifuge radius on the physiology and behavior of a small primate, the squirrel monkey (*Saimiri sciureus*). The studies measured the responses of these primates to centrifugation in a constant acceleration field at different radii (0.9 and 3 m).

## METHODS

Eight adult male squirrel monkeys (*Saimiri sciureus*) weighing 800-1000 g were used in this study. Animals were housed singly in clear Plexiglass cages measuring 51 x 32 x 22 cm. The cages were placed in separate modules on the centrifuges. The modules provided one degree of freedom such that the acceleration vector was always perpendicular to the cage floor. Positive air flow ventilation was provided in each module. Each monkey was visually isolated from the other animals and from the environment external to the module while on the centrifuge. Ambient temperature was held constant at  $23\pm 2^{\circ}\text{C}$  and a 24 hr light-dark cycle (LD12:12) was used throughout. Light intensity was 600 lux during the light period and 0 lux during the dark. Food and water were provided *ad libitum*.

Each animal was implanted with an intraperitoneal biotelemetry transmitter for the measurement of deep body temperature and activity (Mini-Mitter, Model T). The surgery was performed under general inhalational anesthesia (halothane and oxygen) using aseptic technique. A minimum recovery time of two weeks was allowed prior to initiation of experiments.

Two centrifuges of different radii (0.9 m and 3.0 m) were utilized in this study (see Figure 1). A constant field of 1.5 G was generated by each centrifuge. The eight animals were divided into two groups of four. The first group was studied on the 3.0 m centrifuge followed by the 0.9 m centrifuge. The order was reversed for the second group of animals. The monkeys were housed on a stationary centrifuge at 1 G for a minimum of four days prior to the initiation of data collection to allow acclimation to single housing and the cage; based on our previous experience, stable circadian rhythms are seen after a four day adaptation period. A 10 day baseline period at 1 G was followed by 10 days at 1.5 G (18.2 rpm and 33.3 rpm on the 3.0 m and 0.9 m centrifuges respectively). The centrifuge was stopped for 15 minutes each day in order to perform animal care, i.e., feed, water, and change

litter trays. A ten day period of data collection at 1 G followed centrifugation. A minimum of 30 days was allowed between the two studies for each animal.

Daily totals of food and water consumption were recorded. Deep body temperature and activity counts were recorded every five minutes and stored on an IBM XT microcomputer (Dataquest). Time-lapse video recordings (1 frame every 0.1 seconds) were made of representative individuals for observational analysis. Data analysis was performed on Macintosh SE and DEC MicroVax II computers. A sine wave was fitted to each 24 hour segment of the activity and body temperature data. The time of the acrophase (maximum) of the sine wave is the circadian phase; the amplitude of the sine wave is the amplitude of the circadian rhythm. Statistical differences were calculated using an analysis of variance (ANOVA) for repeated measures ( $p \leq 0.05$ ). The effects of centrifugation were compared with 1 G pre- and postcentrifugation recovery.

## RESULTS

Behaviorally, all monkeys tolerated the caging system well. Food and water consumption were within anticipated normal ranges (see below). No distress was noted in any animal during the 10 day pre-centrifugation period. At the onset of 1.5 G, the monkeys were observed to assume an immobile position which lasted several minutes; emesis was frequently noted (approximately 50% of the animals). In some animals emesis occurred within minutes of the start of centrifugation while in other individuals emesis did not occur for an hour or more at 1.5 G. Neither centrifuge size nor run number had an effect on the frequency of emesis. It appeared that, animals that exhibited emesis were more quiescent during the onset of centrifugation than animals that did not. By day 2 of exposure to 1.5 G emesis was rarely observed.

No discernable difference could be noted in the behavior of the monkeys on the 0.9 m centrifuge versus the 3.0 m centrifuge. The animals remained subdued for 2 to 4 days after which their visually observed alertness and activity approached pre-centrifugation levels on both the 0.9 and 3.0 m centrifuges. The return to 1.0 G at the end of 10 days of centrifugation at 1.5 G caused no discernable behavioral changes; no emesis, quiescence, or disorientation was noted on either centrifuge. Complete activity and temperature data sets are available for only 6 animals because of transmitter failures. Averages for each measured variable with standard errors are given for each phase of the study in Table 1.

**Feeding.** As shown in Figure 2 (upper panel), average daily food consumption during the 10 day pre-centrifugation period was similar on both the 0.9 m and 3.0 m centrifuges ( $7.9 \pm 0.3$  and  $7.6 \pm 0.2$  biscuits/day (mean  $\pm$  SE) respectively). During 10 days of centrifugation, food consumption decreased significantly at both radii (to  $4.1 \pm 0.5$  biscuits/day on the 0.9 m centrifuge and to  $5.2 \pm 0.9$  biscuits/day on the 3.0 m centrifuge). During the 10 day post-centrifugation

recovery period, food consumption increased significantly (to  $8.3 \pm 0.3$  (0.9 m) and  $9.2 \pm 0.2$  (3.0 m) biscuits/day).

Daily mean food consumption is plotted vs. day of experiment in Figure 2 (lower panel). As can be seen, at the onset of 1.5 G, food consumption decreased to less than two biscuits/day on both centrifuges. After two days at 1.5 G, food consumption began to increase. The rate of recovery of food consumption at 1.5 G was slower on the 0.9 m centrifuge. On the 3.0 m centrifuge, food consumption had returned towards pre-centrifugation levels by day 6. On the 0.9 m centrifuge, however, food consumption increased towards, but never attained, pre-centrifugation levels over the 10 days at 1.5 G.

**Drinking.** The average daily water consumption on each centrifuge for each of the three phases of the experiment is plotted with standard errors in Figure 3 (upper panel). Water consumption was not significantly different between the two centrifuge runs during the 10 day pre-centrifugation period: the average water consumption was  $136 \pm 7$  ml/day on the 0.9 m and  $148 \pm 11$  ml/day on the 3.0 m centrifuge. During the 10 days of centrifugation, the average water consumption decreased to  $54 \pm 8$  ml/day on the 0.9 m centrifuge ( $p \leq 0.05$ ) and to  $112 \pm 14$  ml/day on the 3.0 m centrifuge (n.s.). There was a gravity by centrifuge radius interaction: water consumption was significantly lower at 1.5 G on the 0.9 m centrifuge than on the 3.0 m centrifuge. As with feeding, during the 10 day post-centrifugation recovery period water consumption increased to slightly more than pre-centrifugation levels, rising to  $155 \pm 8$  (0.9 m) and  $157 \pm 7$  ml/day (3.0 m). Water consumption levels were significantly higher during the post-centrifugation recovery period than during centrifugation at both radii.

Average daily water consumption is plotted vs. day of experiment in Figure 3 (lower panel). At the onset of 1.5 G, water consumption decreased to approximately 22 ml/day on the 0.9 m centrifuge and to approximately 81 ml/day on



the 3.0 m centrifuge. Over a period of several days at 1.5 G, water consumption began to increase toward pre-centrifugation levels. The rate of recovery of water consumption, was slower on the 0.9 m centrifuge than the 3.0 m centrifuge, similar to food consumption. On the 3.0 m centrifuge, water consumption had returned towards mean pre-centrifugation levels by day 6. On the 0.9 m centrifuge, however, water consumption slowly increased towards, but never attained, pre-centrifugation levels over the 10 days at 1.5 G.

**Body temperature.** Core body temperature averaged  $38.5 \pm 0.01^{\circ}\text{C}$  and  $38.7 \pm 0.01^{\circ}\text{C}$  during the 10 day pre-centrifugation period on the 0.9 m and the 3.0 m centrifuges respectively. As can be seen in Figure 4 (upper panel), no significant changes were observed in mean body temperature averaged over the 10 day 1.0 G baseline, 1.5 G centrifugation ( $38.6 \pm 0.01$ ;  $38.6 \pm 0.02$ ), and 1.0 G recovery periods ( $38.5 \pm 0.02$ ;  $38.6 \pm 0.02$ ) on either centrifuge. This lack of influence of the hypergravity environment on the daily average body temperature is evident in Figure 4 (lower panel) where the average daily body temperature is plotted vs. day of experiment.

Figure 5 (upper panel) depicts the average amplitude of the circadian temperature rhythm for each phase of the experiment. The amplitude of the circadian temperature rhythm averaged  $1.3 \pm 0.02^{\circ}\text{C}$  on the 0.9 m centrifuge and  $1.3 \pm 0.06^{\circ}\text{C}$  on the 3.0 m centrifuge during the 10 day pre-centrifugation period. During centrifugation this decreased significantly (to  $0.9 \pm 0.07^{\circ}\text{C}$  on the 0.9 m centrifuge and to  $1.1 \pm 0.05^{\circ}\text{C}$  on the 3.0 m centrifuge). As was seen in water consumption, there was a significant gravity by radius interaction; temperature amplitude was decreased to a greater extent at hyperG levels on the smaller radius centrifuge. The daily temperature amplitude is plotted vs. day of experiment in Figure 5 (lower panel). On the 3.0 m centrifuge, the amplitude of the temperature rhythm recovered to pre-centrifugation levels by day 6. In contrast, on the 0.9 m

centrifuge, amplitude remained decreased. During the 10 day post-centrifugation period, average amplitudes of the temperature rhythm returned to near baseline levels ( $1.2 \pm 0.04^{\circ}\text{C}$  and  $1.3 \pm 0.05^{\circ}\text{C}$  on the 0.9 m and 3.0 m centrifuges respectively). This represented a significant increase from amplitude during hyperG.

Mean circadian phase position of the temperature rhythm is shown in Figure 6 (upper panel) for the pre-centrifugation, hypergravity, and post-centrifugation sections of the experiment. During the 10 day baseline period the average phase of the temperature rhythm occurred at  $15.0 \pm 0.04$  hours and  $14.9 \pm 0.04$  hours on the 0.9 m centrifuge and 3.0 m centrifuge respectively. This was delayed to  $15.3 \pm 0.18$  hours on the 0.9 m centrifuge and to  $15.1 \pm 0.13$  hours on the 3.0 m centrifuge. The delay was significant only at the smaller radius. There was no significant difference between the phases on either centrifuge at 1.5 G. No changes in the phase of the circadian temperature rhythm was noted upon return to 1.0 G on either centrifuge. However, as can be seen in Figure 6 (lower panel), the average time course of the response to hypergravity differed between the two centrifuges. On the smaller centrifuge, the marked, transient phase delay to  $16.6 \pm 0.5$  hours on day 1 was followed by recovery on day 2. In contrast, on the 3.0 m centrifuge, a smaller biphasic phase delay/advance occurred over several days at 1.5 G.

**Activity.** Mean daily activity counts for each section of the experiment are shown ( $\pm$  standard errors) in Figure 7 (upper panel). On the 0.9 m centrifuge activity averaged  $45.4 \pm 3.9$  counts per 5 minute interval during the 10 day pre-centrifugation baseline; this value decreased to  $36.2 \pm 3.6$  during centrifugation, and, in contrast to the feeding and drinking values, showed a further decrease during the postcentrifugation period ( $25.4 \pm 2.1$ ). The corresponding values for the 3.0 m centrifuge were:  $36.1 \pm 1.1$ ,  $28.7 \pm 2.4$ , and  $30.8 \pm 1.2$ . The daily average activity levels are plotted vs. day of experiment in Figure 7 (lower panel). The

great decrease in activity that occurred at the onset of centrifugation is evident, as is the subsequent recovery although pre-centrifugation activity levels were not attained during the postcentrifugation recovery period in either group.

The average amplitude of the activity rhythm is shown in Figure 8 (upper panel) for each section of the experiment. Once again, the animals on the 0.9 m centrifuge showed a steady decrease across all three phases of the experiment ( $56.4 \pm 5.2$ ;  $38.0 \pm 4.1$ ;  $29.4 \pm 3.1$ ) while results from the larger radius centrifuge showed a decrease during centrifugation and a recovery back towards baseline ( $45.3 \pm 1.5$ ;  $32.6 \pm 3.2$ ;  $39.6 \pm 1.4$ ). Both of these observations are evident in Figure 8 (lower panel), which plots the daily mean activity amplitude vs. day of experiment.

Average phase of the activity rhythm for each section of the experiment is shown in Figure 9 (upper panel). As can be seen, the phase of the activity rhythm evidenced an advance from baseline to centrifugation on both the 0.9 m ( $14.1 \pm 0.3$  hours to  $13.6 \pm 0.4$  hours) and 3.0 m ( $14.6 \pm 0.2$  hours to  $14.1 \pm 0.4$  hours) centrifuges with a subsequent delay during the postcentrifugation recovery period ( $14.0 \pm 0.4$  hours on the 0.9 m and  $15.0 \pm 0.2$  hours on the 3.0 m). Average daily phase is shown vs. day of experiment in Figure 9 (lower panel).

## DISCUSSION

The study was designed to examine the potential influence of centrifuge radius on the responses of the primate feeding, drinking, activity, and thermoregulatory systems to the hyperdynamic environment. At the time of this study, the maximum radius centrifuge which could be accommodated in a race in the pressurized laboratory module was 0.9 m, so we examined the responses of these systems to 1.5 G on centrifuges of radii of 0.9 m and 3.0 m. Current estimates are that a 1.25 m radius centrifuge can be mounted in the end cone of a node so results must be extrapolated.

Numerous physiological changes occur during exposure of animals to increased acceleration fields produced by means of centrifugation [7]. Typically these responses are triphasic [4] in that an initial response is followed by a recovery period and then acclimation. This typical response was seen in this study; initial exposure to centrifugation resulted in a decrease in most measured parameters. An increase back towards baseline was generally seen, although recovery to pre-centrifugation levels was not always attained. In all three variables where significant differences were noted between the two centrifuge radii, the initial decrease was always greater and the recovery slower on the smaller radius centrifuge.

The principal physical change in orbiting vehicles is the removal of the effects of Earth gravity. Understanding this phenomenon is of critical importance to the continuing development of gravitational biology. The spacecraft environment also has other factors that may modify biological function (i.e., solar and cosmic radiation, illumination schedule, forces and materials produced in the Space Station such as noise, vibration, or environmental contaminants). These secondary factors may produce independent biological effects or may modify the effects of weightlessness. An interaction of the effects of ionizing radiation and

gravitational fields has been demonstrated in rats [2,3] and in plants and microorganisms in orbiting satellites [5]. In short term orbital experiments these extraneous factors may not significantly affect results; however, in the protracted exposure anticipated with the Space Station Freedom, their cumulative effects may seriously interfere with research. It is of critical importance that any influence of these extraneous factors upon biological experiments in the Space Station be identified so they can be separated and not confused with the effects of weightlessness. Provision must therefore be made for a suitable control as a part of Space Station experiments. This can only be fulfilled by an on-board centrifuge operating at 1 G. If the only biologically significant factor in the Space Station environment is weightlessness, the responses of on-board 1 G controls should be the same as those exhibited by equivalent ground-based controls. The use of on-board 1 G-controls on the Space Station should be continued until all variables in the Space Station environments are identified and determined not to have interfering biological effects

The ratio of specimen stature ("height") to the radius of rotation is also important because of potential interference from "head-to-foot" G-gradients in experimental subjects. This complication has been deliberately avoided by providing a large radius of rotation. Due to the dimensional limits upon centrifuge size in Space Station it is imperative that ground-based research be initiated to identify the biological effects of head-to-foot G-gradients. Such information may provide suitable correction factors, or indicate the maximum G-gradient that will not interfere with experimentation. The latter may limit the numbers of species (on the basis of stature) amenable to centrifugation on the Space Station.

Ground based studies [1] indicate that there is a time-intensity summation for gravitational effects, so that brief interruptions do not greatly affect the results of a biological experiment. Experiments which involve a daily 15 minute interruption in

centrifugation (about 1% of a day) will yield results similar to those from experiments in which the centrifugation is continuous, but at 99% of the field strength. The variability in biological response to ground-based acceleration is such that this difference could not be detected. However, this relationship may not apply in a Space Station where the suspension of centrifugation will involve weightlessness, potentially producing a disorientation that may induce separate and significant biological effects.

Our results demonstrate that centrifuge radius does appear to have an effect on several parameters of the response to hyper-G. The initial response to hypergravity was generally greater on the smaller radius centrifuge and recovery back to baseline was usually faster after centrifugation at the larger radius.

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## FIGURE LEGENDS

Table 1. Average daily values for each measured variable during each 10 day phase of the study are given with standard errors. Units are as follows: feeding is biscuits/24 hr; drinking is ml/24 hr; body temperature mean and amplitude are degrees centigrade; body temperature phase is hours; activity mean and amplitude are counts/5 minute interval; and activity phase is hours.

Figure 1. Line drawings of the 0.9 m radius (left) and the 3.0 m radius (right) centrifuge used in this study. Modules on both centrifuges provided one degree of freedom such that the acceleration vector was always perpendicular to the cage floor.

Figure 2. Upper panel: The average number of biscuits consumed per day is plotted (+ se) vs. phase of experiment for each centrifuge. At either radius of rotation, the level of food consumption during 1.5 G is significantly lower than that of either of the 1.0 G periods.

Lower panel: Average number of biscuits consumed is plotted ( $\pm$  se) vs. day of the experiment for each centrifuge. The decrease at the onset of 1.5 G, as well as the return toward baseline levels can be seen. Recovery was faster on the larger radius centrifuge.

Figure 3. Upper panel: The average amount of water consumer per day is plotted (+ se) vs. phase of the experiment for each centrifuge. Water consumption decreased to a significantly lower level during hyperG on the smaller centrifuge than on the larger. Water consumption levels increased significantly during the postcentrifugation period.

Lower panel: Average water consumption is plotted ( $\pm$  se) vs. day of the experiment for each centrifuge. The decrease at the onset of centrifugation as well as the faster recovery on the larger centrifuge are evident.



Figure 4. Upper panel: Average mean body temperature is shown as in Fig. 2 (upper).

Lower panel: Daily mean body temperature is shown as in Fig. 2 (lower).

Figure 5. Upper panel: Average amplitude of the circadian temperature rhythm is shown as in Fig. 2 (upper). HyperG had a significant effect on temperature rhythm amplitude. This difference was compounded by centrifuge radius; temperature amplitude decreased to a greater extent on the smaller centrifuge.

Lower panel: Daily mean circadian temperature amplitude is shown as in Fig. 2 (lower).

Figure 6. Upper panel: Average phase of the temperature rhythm is plotted as in Fig. 2 (upper). The phase of the temperature rhythm was significantly delayed on the smaller centrifuge by hypergravity.

Lower panel: Daily mean phase of the temperature rhythm is plotted as in Fig. 2 (lower).

Figure 7. Upper panel: Average mean activity levels are plotted as in Fig. 2 (upper).

Lower panel: Daily mean activity levels are shown as in Fig. 2 (lower).

Figure 8. Upper panel: Average amplitude of the circadian activity rhythm is shown as in Fig. 2 (upper).

Lower panel: Daily mean circadian activity amplitude is plotted as in Fig. 2 (lower).

Figure 9. Upper panel: Average phase of the activity rhythm is plotted as if Fig. 2 (upper).

Lower panel: Daily mean phase of the activity rhythm is plotted as in Fig. 2 (lower).

TABLE 1

	PRE 1.0G			HYPER 1.5G			POST 1.0G		
	1.8 M	6.0 M	1.8 M	1.8 M	6.0 M	6.0 M	1.8 M	6.0 M	6.0 M
CENTRIFUGE									
FEEDING	7.9+0.4	7.6+0.2	4.1+0.5	5.2+0.9	8.3+0.3	9.2+0.2			
DRINKING	136+7	148+11	543+8	112+14	155+8	157+7			
BODY TEMPERATURE									
MEAN	38.5+0.02	38.7+0.02	38.6+0.03	38.6+0.02	38.5+0.02	38.6+0.02			
AMPLITUDE	1.3+0.02	1.3+0.05	0.9+0.07	1.0+0.05	1.2+0.04	1.3+0.05			
PHASE	15.0+0.04	14.9+0.04	15.34+0.18	15.11+0.13					
ACTIVITY									
MEAN	45.4+3.9	36.1+2.9	36.2+3.6	28.7+2.4	25.4+2.1	30.8+2.2			
AMPLITUDE	56.4+5.2	45.3+1.6	38.0+4.1	32.6+3.2	29.4+3.1	39.6+1.4			
PHASE	14.1+0.3	14.6+0.2	13.6+0.4	14.1+0.4	14.0+0.4	15.0+0.2			

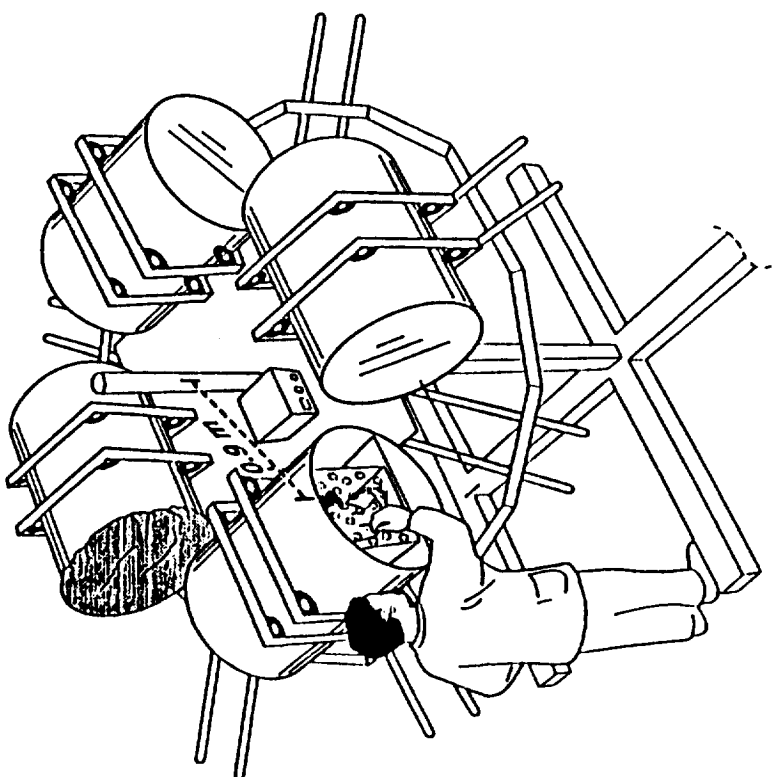
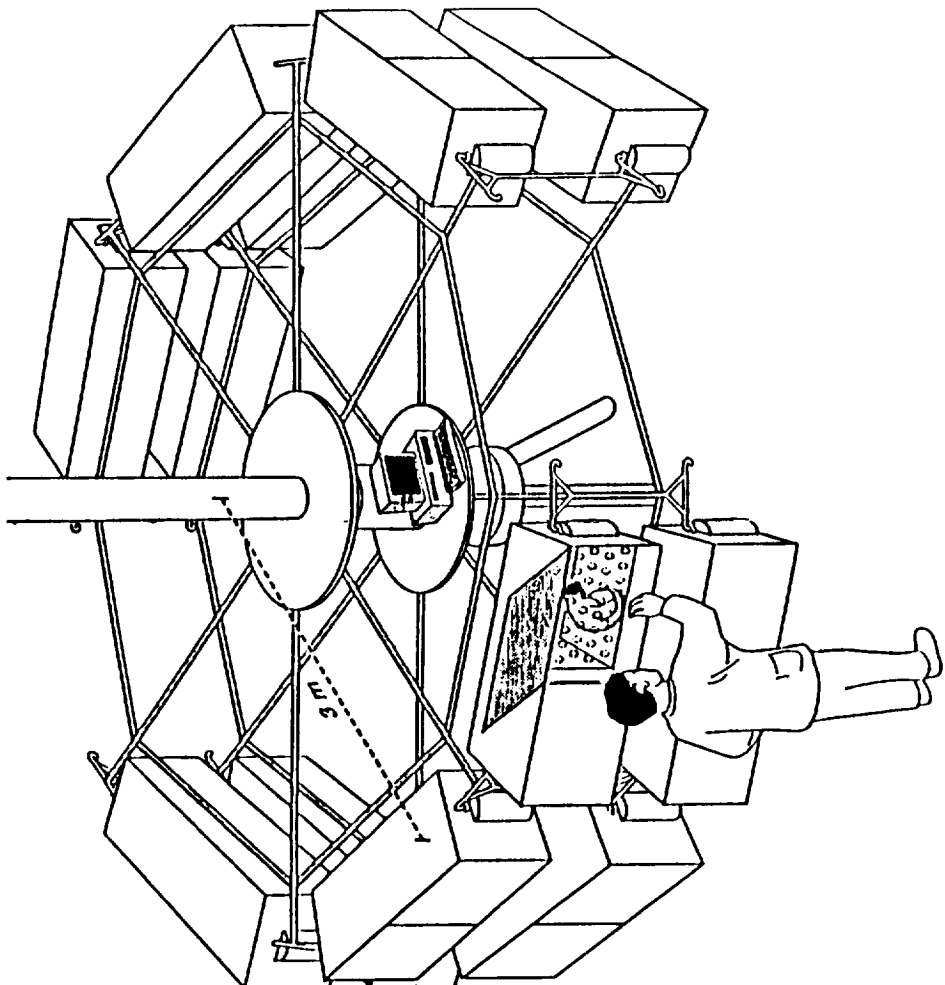


FIG. 1

### 3.0M vs 0.9M FOOD CONSUMPTION

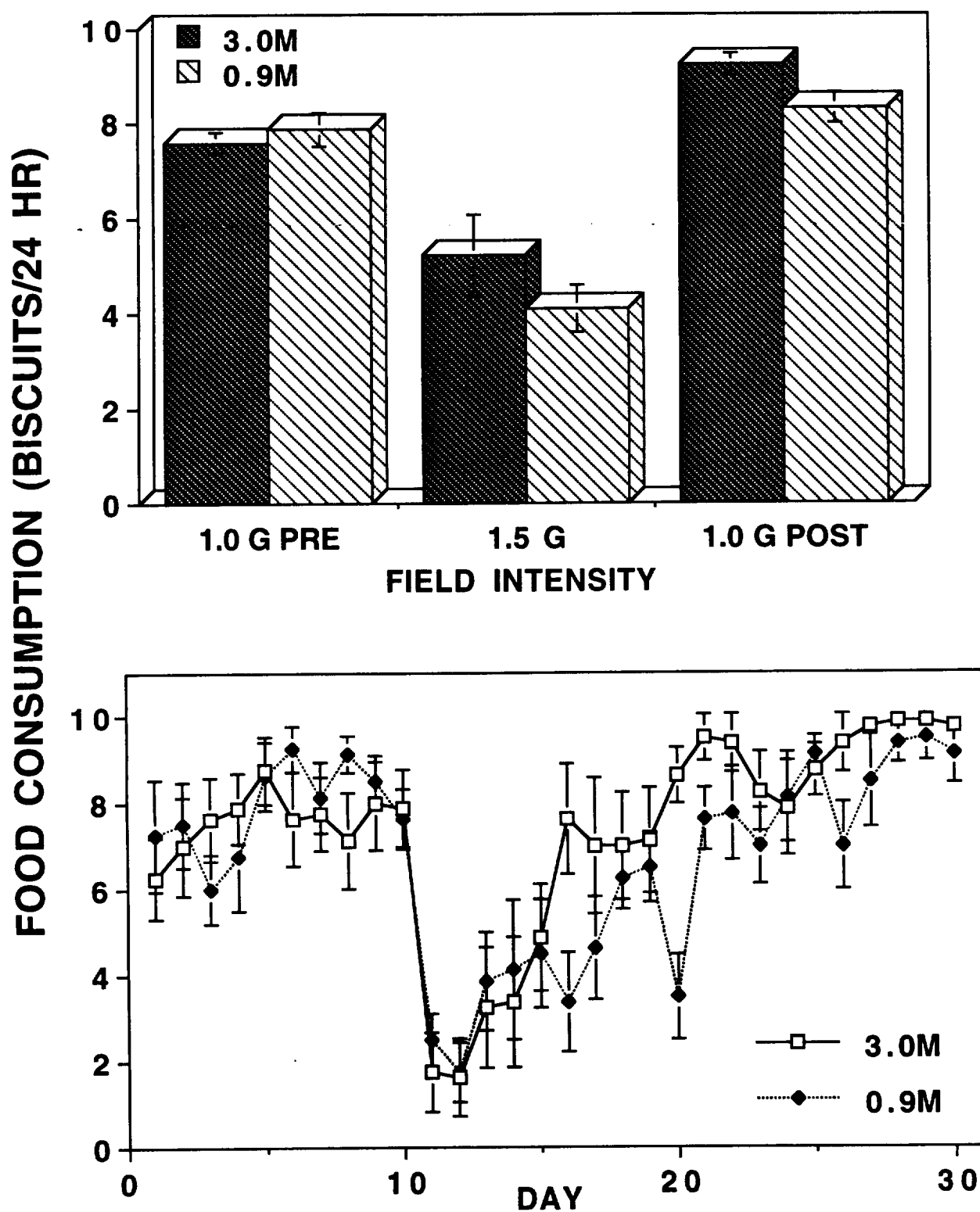


FIG. 2

### 3.0M vs 0.9M WATER CONSUMPTION

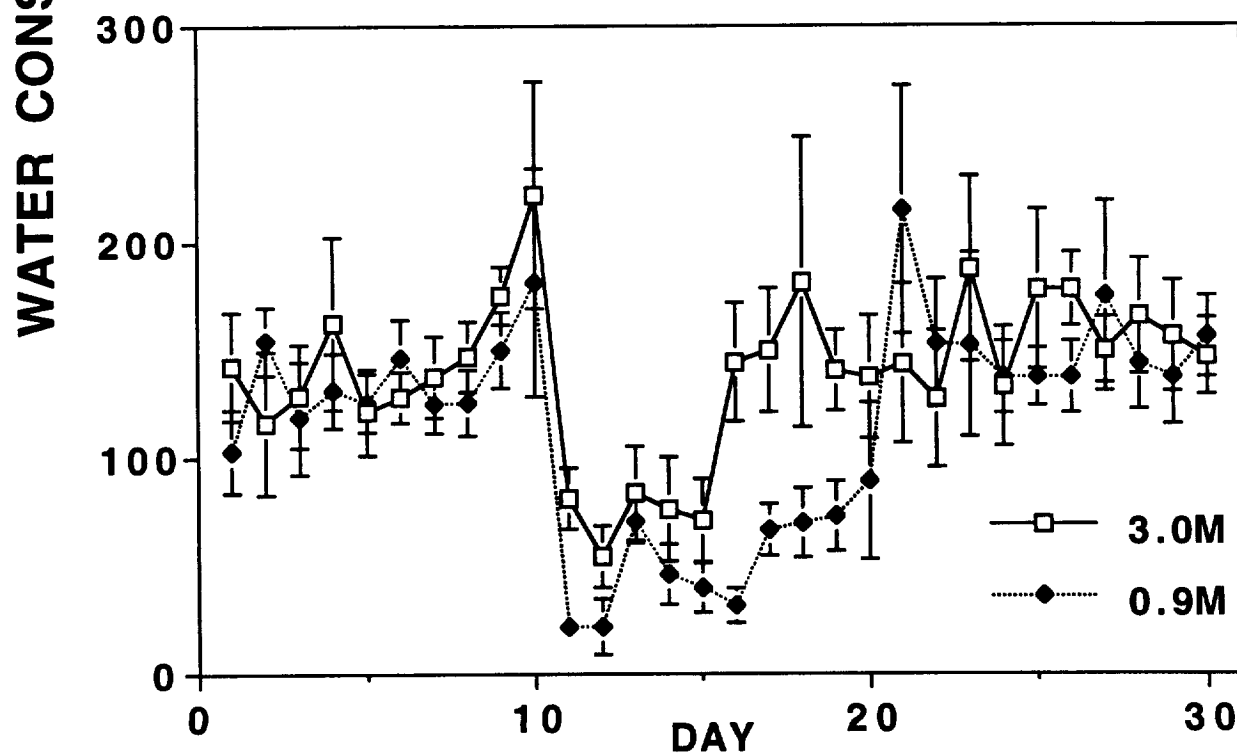
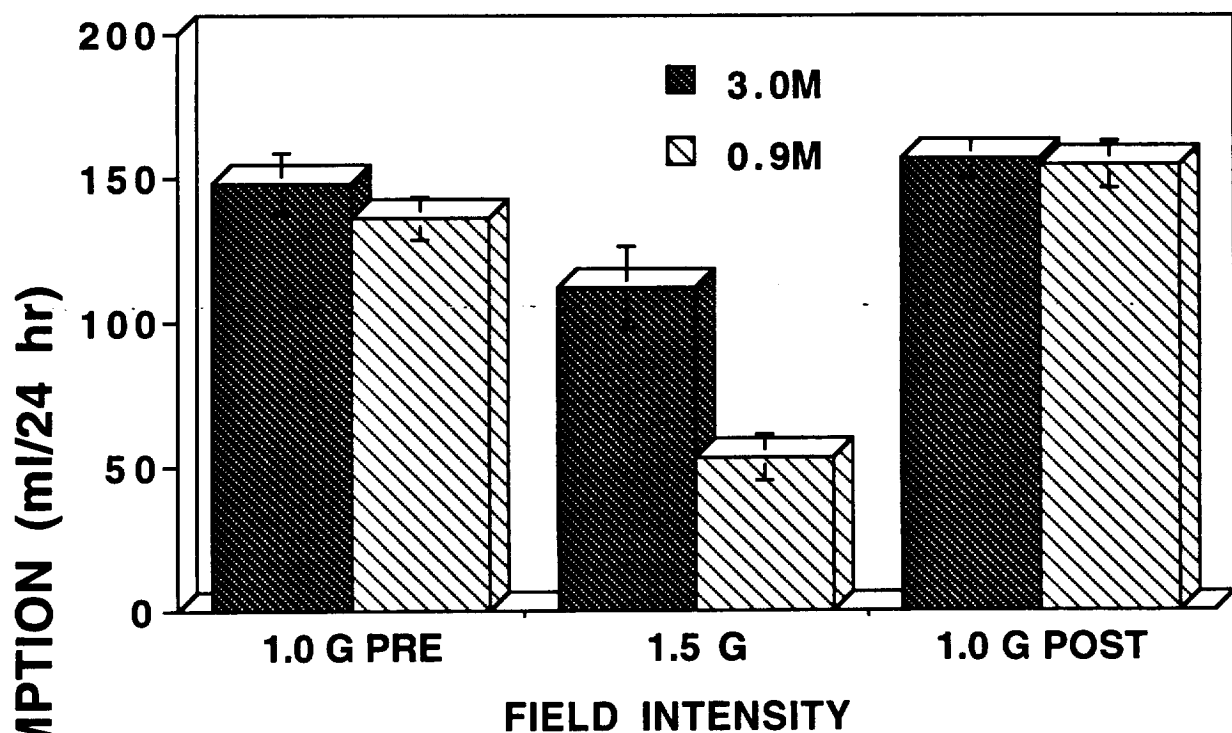


FIG. 3

### 3.0M vs 0.9M TEMPERATURE MEAN

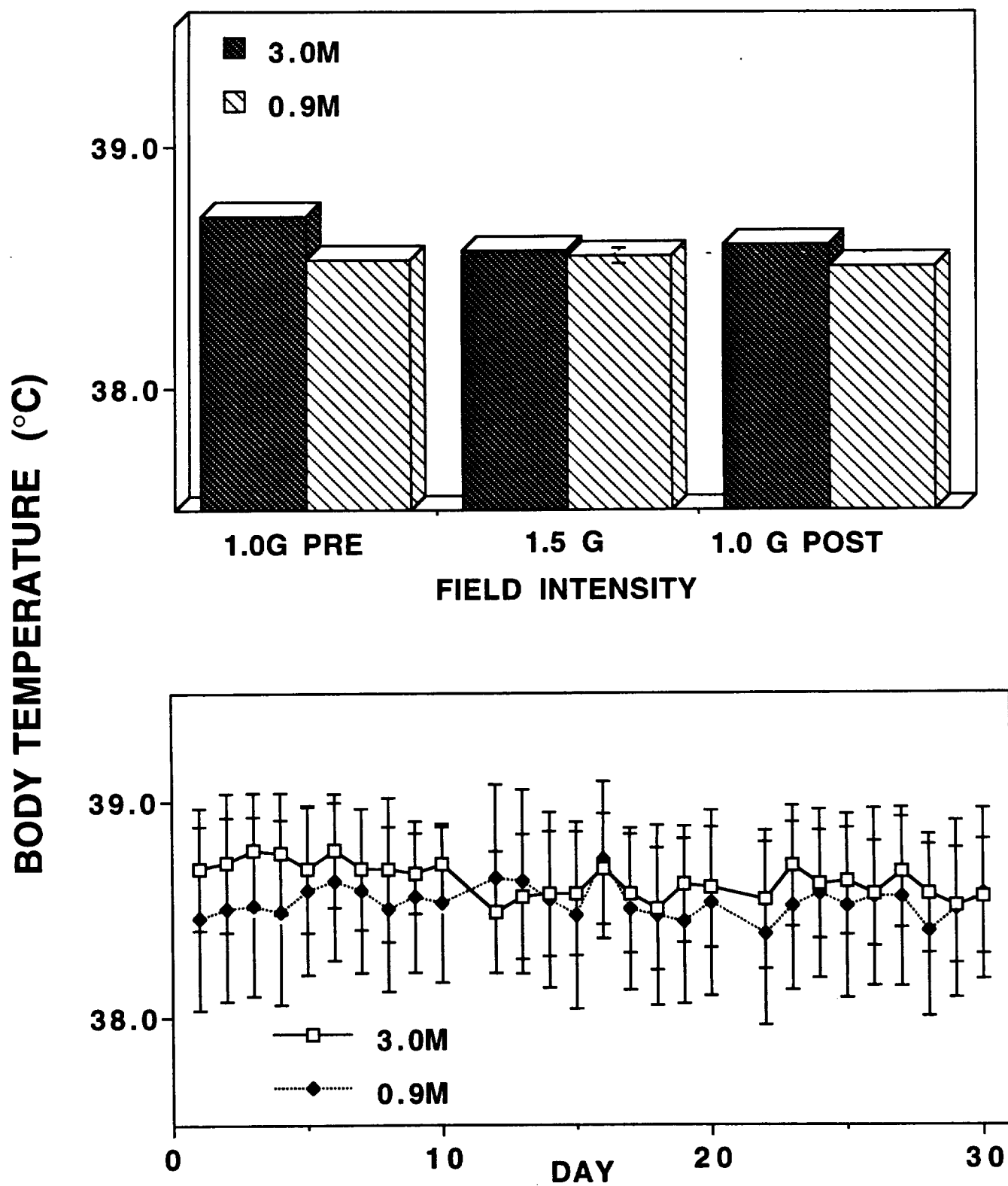


FIG. 4

## 6.0M vs 1.8 M TEMPERATURE AMPLITUDE

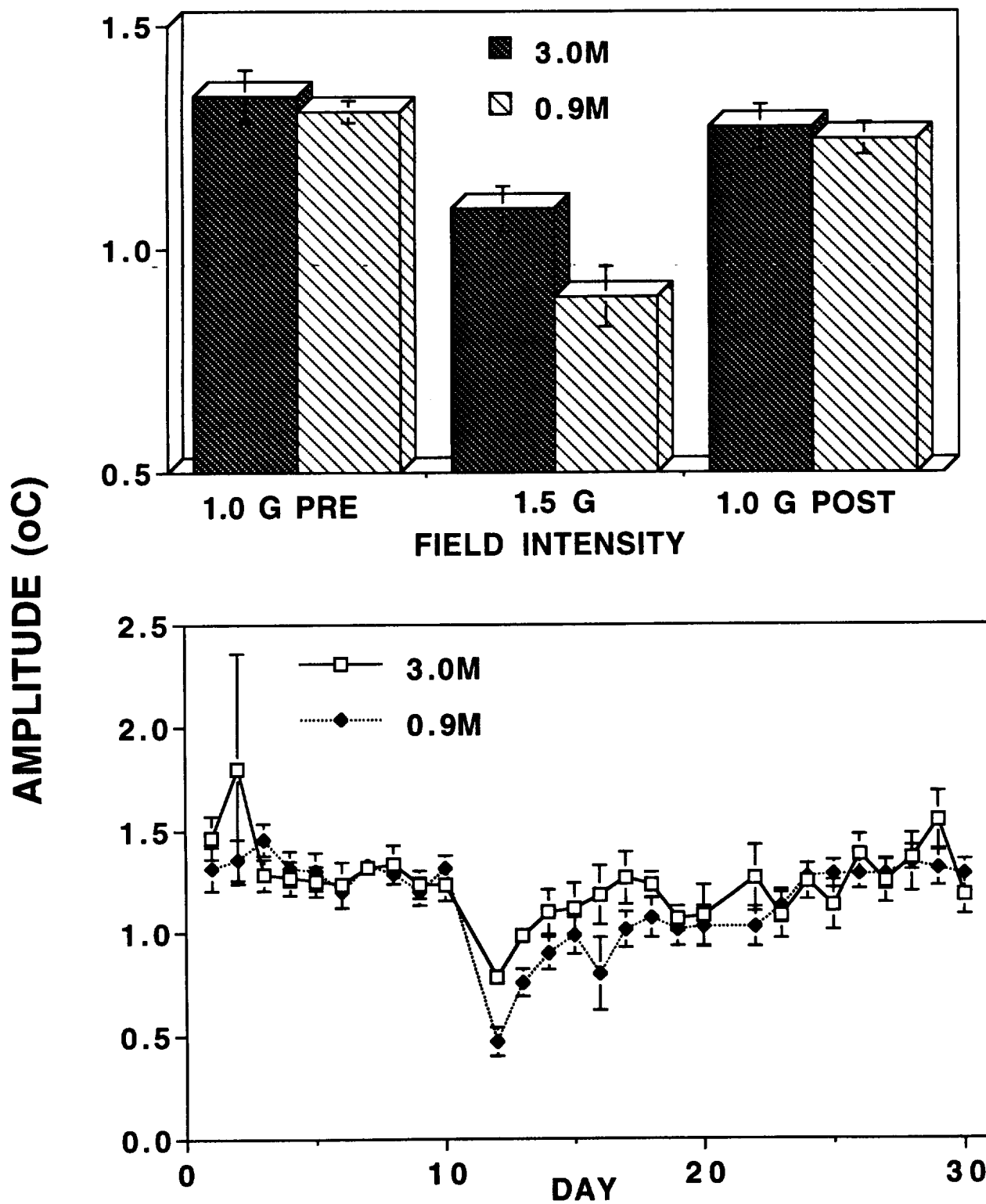


FIG. 5

### 3.0M vs 0.9M TEMPERATURE PHASE

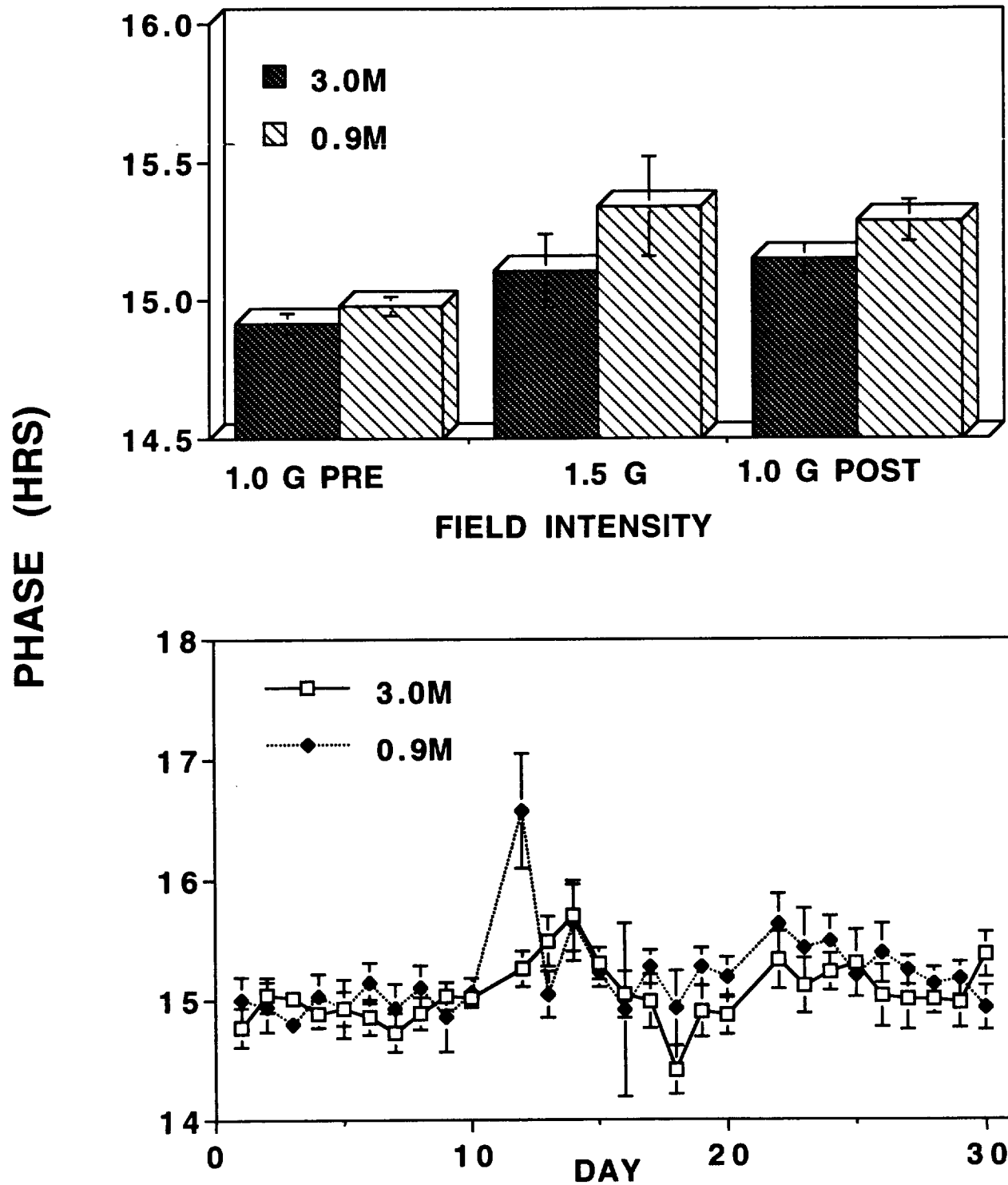
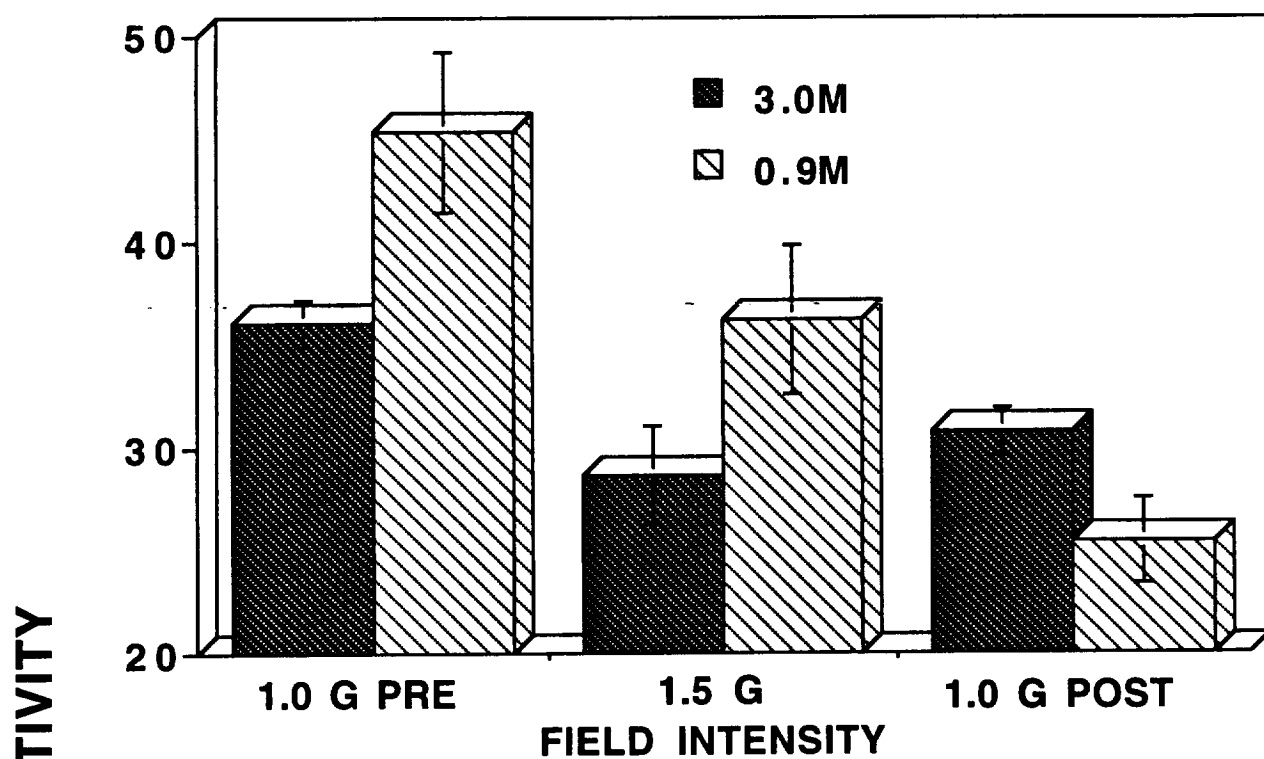


FIG. 6



### 3.0M vs 0.9M ACTIVITY MEAN



MEAN ACTIVITY

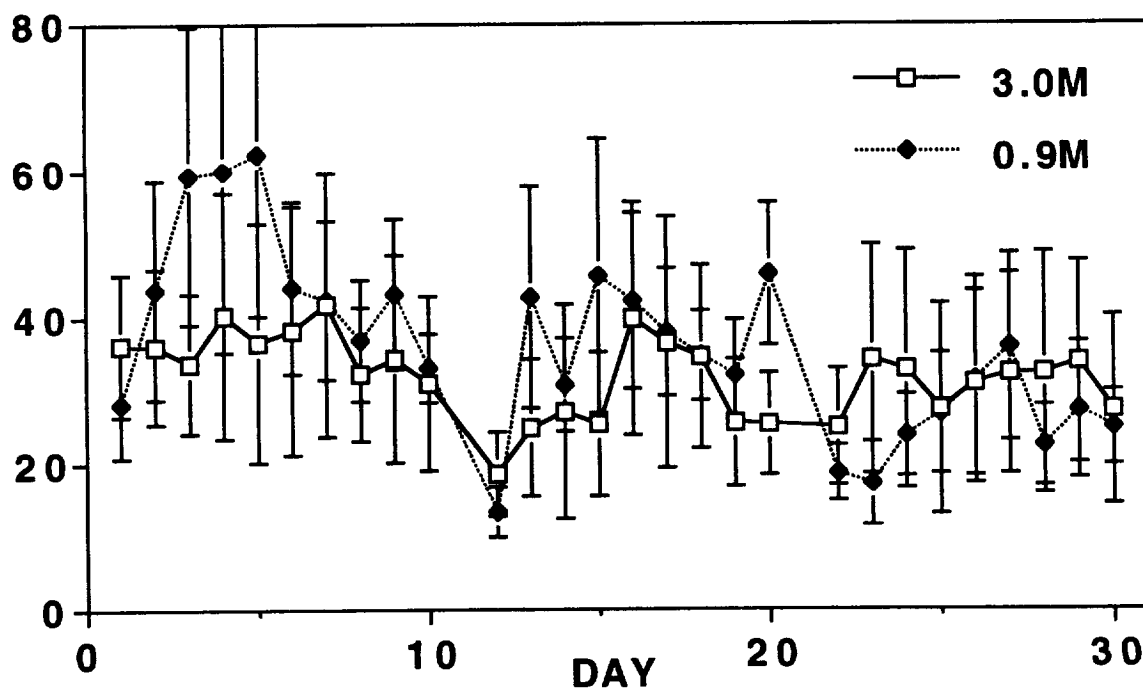


FIG. 7

### 3.0M vs 0.9 M ACTIVITY AMPLITUDE

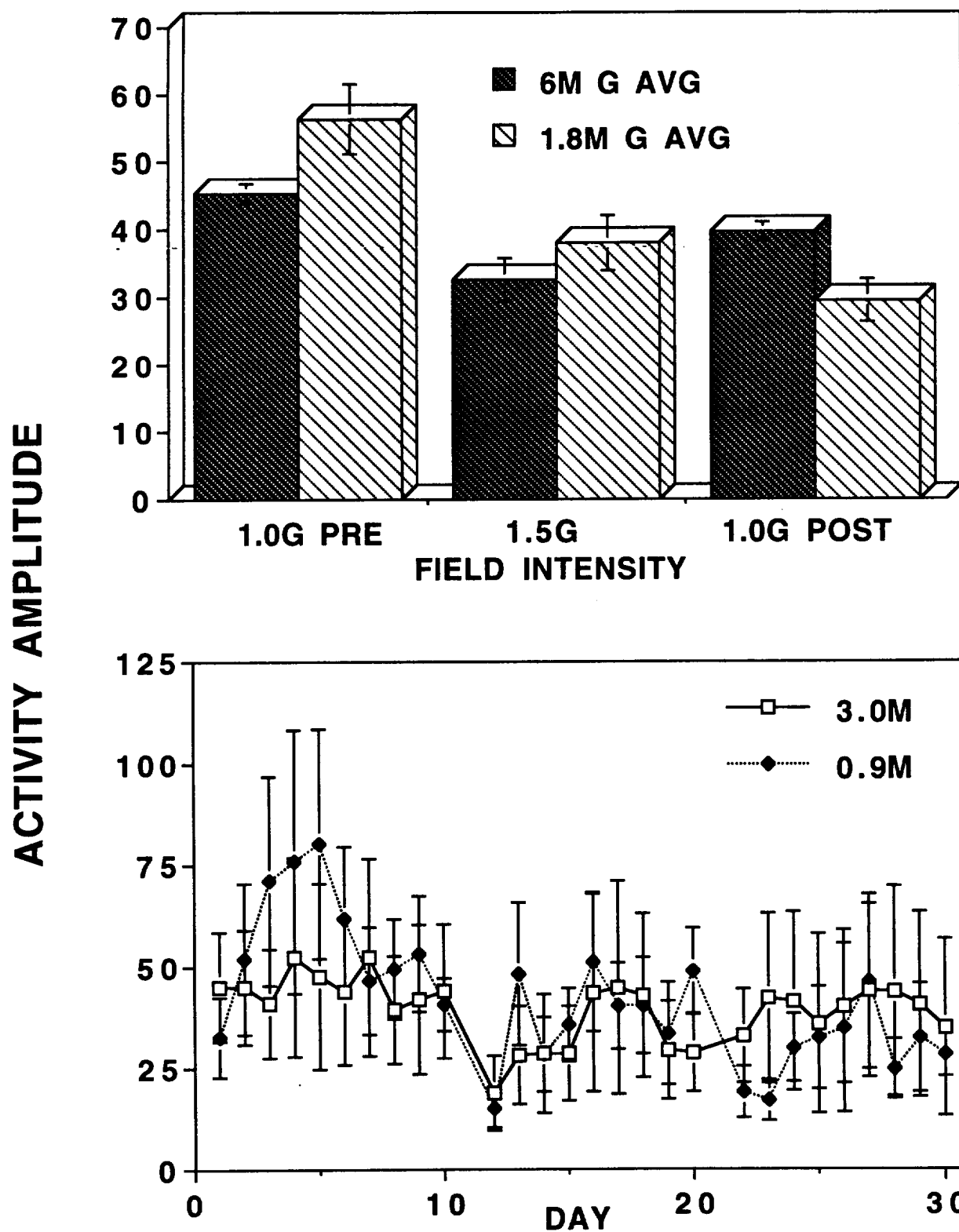


FIG. 8

### 3.0M vs 0.9M ACTIVITY PHASE

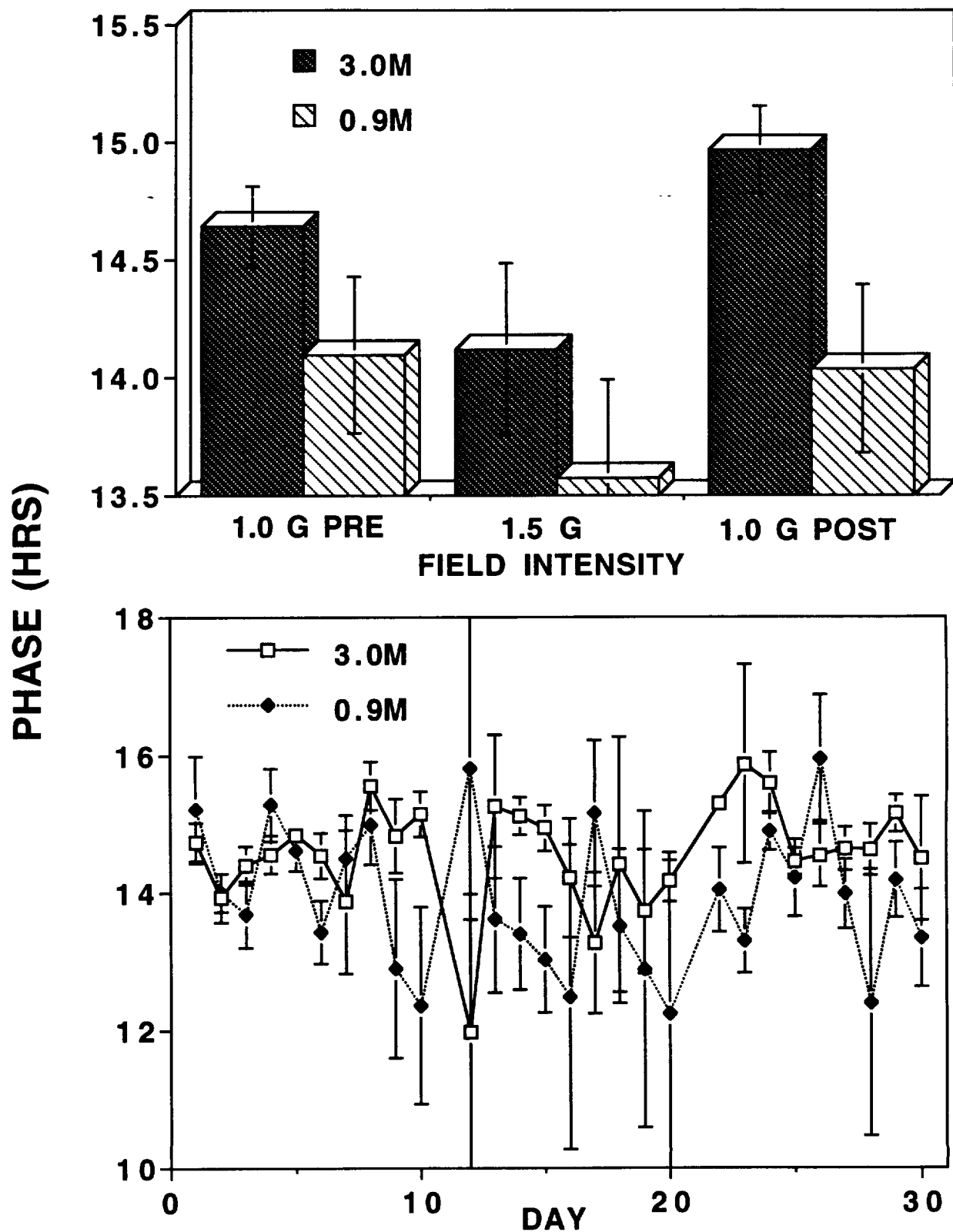


FIG. 9